

## *Eimeria praecox* infection ameliorates effects of *Eimeria maxima* infection in chickens

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### Abstract

The effect of *Eimeria praecox* on concurrent *Eimeria maxima* infection was studied in susceptible chickens. Clinical signs of coccidiosis were assessed in single *E. praecox* or *E. maxima* infections and compared to dual infection with both *Eimeria* species. Groups infected solely with  $10^4$  *E. maxima* oocysts displayed weight gains that were 48% of weight gain in uninfected controls. Weight gain in chickens infected only with  $10^4$  *E. praecox* oocysts was 90% of uninfected controls. Average weight gain in chickens infected with both *E. maxima* and *E. praecox* was 79% of controls, and showed no significant difference ( $P > 0.05$ ) from weight gain in *E. praecox*-infected chickens. Feed utilization (feed conversion ratio, FCR) in chickens infected with both species showed no significant difference ( $P > 0.05$ ) from FCR in non-infected controls or chickens infected with *E. praecox* alone; all showing a significant difference ( $P < 0.05$ ) from FCR in chickens infected solely with *E. maxima*. Although *E. praecox* did not appear to have a negative effect on weight gain and FCR, it did cause a significant decrease in serum carotenoids. Analysis of oocysts excreted by chickens during dual infection showed little effect of *E. praecox* on *E. maxima* oocyst production.

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**Keywords:** *E. praecox*; *E. maxima*; Co-infection; Clinical effects

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### 1. Introduction

*Eimeria praecox* is generally regarded as being less pathogenic than other species of *Eimeria*, such as *Eimeria acervulina* and *Eimeria maxima*. For instance, intestinal lesions typical of *E. acervulina* and *E. maxima* are not observed during *E. praecox* infection, the only effect being a watery or mucoid exudate (Gore and Long, 1982). Also, significant weight gain depression is only observed at fairly high challenge doses (Long, 1968; Gore and Long, 1982; Jorgensen et al., 1997; Williams, 1998). The basis of lower pathogenicity of *E.*

*praecox* is unknown, but may be due to an extremely short pre-patent period (84 h) (Gore and Long, 1982; Williams, 1998, 2001; Johnston et al., 2001) or the particular epithelial cells that are invaded in the upper-middle intestine (Johnston et al., 2001). Observations by our group and others have found *E. praecox* in a high percentage of litter samples from commercial broiler operations (Salisch, 1990; McDougald et al., 1997; Jenkins et al., 2006; Morris et al., 2007; Haug et al., 2008). These samples often contain multiple *Eimeria* species, including *E. acervulina* and *E. maxima* (Jenkins et al., 2006; Morris et al., 2007; Haug et al., 2008). Competition for cells in the intestinal epithelia probably occurs between *E. praecox* and either *E. maxima* or *E. acervulina* because of the close proximity of regions of the gut that each species invades. A recent

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study designed to model *E. praecox* and *E. maxima* infections provides some evidence for co-infection of the upper-middle small intestine, but in different sites of the villus (Johnston et al., 2001). However, these authors did not conduct co-infection studies to validate modeling of dual infections. The practical implication of one *Eimeria* species influencing the invasion and development of another *Eimeria* species is that the efficacy of live oocyst vaccines containing a mixture of different species of *Eimeria* may be affected. The purpose of the present study was to determine the effect of *E. praecox* infection on clinical parameters associated with a co-infection with *E. maxima*.

## 2. Materials and methods

### 2.1. Parasites

*E. maxima* (strain Arkansas1) and *E. praecox* (strain North Carolina 2) were derived from field samples, single oocyst-isolated, and propagated every 2–3 months in susceptible chickens using standard procedures (Ryley et al., 1976). The purity of both strains was confirmed by species-specific polymerase chain reaction (PCR) based on ITS1 rDNA sequence (Jenkins et al., 2006).

### 2.2. Infection studies

The effect of *E. praecox* on *E. maxima* infection was carried out by infecting susceptible 4-week-old male Sexsall chickens with either  $10^4$  *E. maxima* oocysts, or  $10^4$  *E. praecox* oocysts, or a mixture of  $10^4$  *E. maxima* and  $10^4$  *E. praecox* oocysts. Individual treatment groups consisted of 15 chickens separated into 3 sub-groups each containing 5 chickens/sub-group. The entire study was repeated twice for a total of three trials. All chickens were assigned to groups on the basis of weight prior to infection using standard randomization methods (Gardiner and Wehr, 1950). On day 7 post-inoculation, all chickens were bled and then killed by cervical dislocation and necropsied for determining upper and middle intestinal lesion scores using standard procedures (Johnson and Reid, 1970). Body weights were obtained for all individual chickens to allow calculation of weight gain during the infection period. Feed conversion ratio (FCR) was calculated for each sub-group of chickens by dividing total feed consumed by total weight gain during the infection period. Serum was prepared from all blood samples, and assayed for carotenoid levels using standard procedures (Allen, 1992). The entire fecal material excreted by chickens in each sub-group was collected between days 3 and 7 post-infection and processed for total *Eimeria*

oocysts using standard procedures with a few modifications (Conway and McKenzie, 2007). In brief, feces from all sub-groups were hydrated with water and then mixed for 1 min at medium speed setting on a Waring mixer (Model 37BL84, New Hartford, CT). A sub-sample was then processed for *Eimeria* oocysts using standard flotation in 1 M sucrose and centrifugation at  $2000 \times g$  for 10 min at 4 °C. *Eimeria* oocysts were collected from the top layer, diluted 10-fold with water, centrifuged at  $2000 \times g$  for 10 min at 4 °C, and suspended in 1.0 ml deionized water. The purified oocysts were examined by microscopy at 400 $\times$  magnification to estimate the percentage of *E. maxima* (31  $\mu\text{m} \times 21 \mu\text{m}$ ) and *E. praecox* (21  $\mu\text{m} \times 17 \mu\text{m}$ ) oocysts, which are distinguishable from each other by size. Oocyst concentrations were determined by counting on a hemocytometer, and the total number of oocysts excreted by each sub-group was calculated by multiplying the concentration of oocysts recovered from a known amount of fecal material times the total fecal material collected over 3–7 days post-infection. Oocyst production/chicken was calculated by dividing the total oocysts produced by the number of chickens in each sub-group. A sample of *Eimeria* oocysts recovered from each sub-group was processed for total DNA using standard procedures (Jenkins et al., 2006), except that DNA was extracted using a commercial MiniDNA kit (Qiagen, Valencia, CA). Oocyst DNA was subjected to ITS1 PCR using primers specific to either *E. praecox* or *E. maxima* in order to compare species composition as assessed by microscopy.

### 2.3. Statistical analysis

Body weight gain, intestinal lesion scores, feed conversion ratios, and serum carotenoid levels were compared between treatment groups using Duncan's multi-variate analysis (SAS Institute, Inc., Cary, NC). Mean *E. praecox* and *E. maxima* oocyst production for all three studies was calculated from the means of three sub-groups in each study, and compared between treatment groups using Mann–Whitney non-parametric statistics (GraphPad InStat Software, San Diego, CA). Mean values were calculated as an average of each parameter for all three trials, and significant differences between groups were noted if  $P \leq 0.05$ .

## 3. Results

### 3.1. Weight gain

Average weight gain in groups infected solely with *E. maxima* oocysts was 48% of weight gain in non-

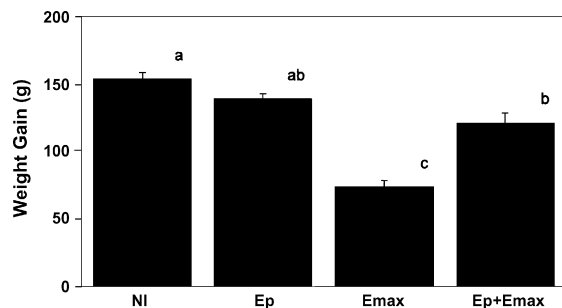


Fig. 1. Mean weight gain over 7 days post-inoculation in chickens infected solely with  $10^4$  *Eimeria praecox* (Ep) oocysts or  $10^4$  *Eimeria maxima* (Emax) oocysts or with  $10^4$  each *E. praecox* and *E. maxima* (Ep + Emax). NI, non-infected controls. Data expressed as mean + S.E.M. of three individual studies. Duncan statistical analysis results are indicated by lower case letters above histogram—no significant difference ( $P > 0.05$ ) between groups that share a letter.

infected controls (Fig. 1). Average weight gain was 90% of non-infected controls in chickens infected with *E. praecox* alone and 79% of non-infected controls in chickens inoculated with both *E. maxima* and *E. praecox* (Fig. 1). Although weight gains were less in single *E. praecox* or dual *E. praecox* and *E. maxima* infection compared to uninfected controls, they showed a significant ( $P < 0.05$ ) increase relative to weight gain in single *E. maxima*-infected chickens.

### 3.2. Feed conversion ratios

Average FCRs in non-infected controls were  $2.5 \pm 0.1$ , whereas FCR in groups infected solely with *E. maxima* oocysts increased to  $4.0 \pm 0.5$  (Fig. 2). Groups infected with *E. praecox* alone showed average FCR equal to  $2.6 \pm 0.1$ , whereas groups infected with

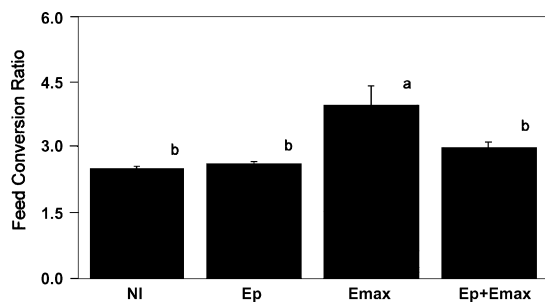


Fig. 2. Mean feed conversion ratio (FCR) over 7 days post-inoculation in chickens infected solely with  $10^4$  *E. praecox* (Ep) oocysts or  $10^4$  *E. maxima* (Emax) oocysts or with  $10^4$  each *E. praecox* and *E. maxima* (Ep + Emax). NI, non-infected controls. Data expressed as mean + S.E.M. of three individual studies. Duncan statistical analysis results are indicated by lower case letters above histogram—no significant difference ( $P > 0.05$ ) between groups that share a letter.

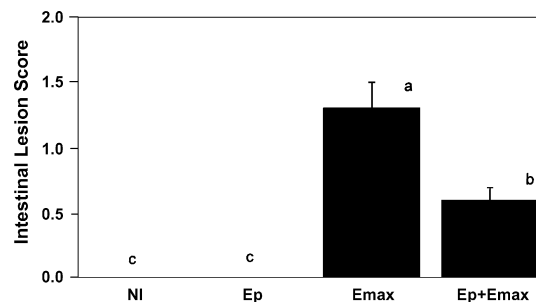


Fig. 3. Mean intestinal lesion scores in middle-upper intestine at 7 days post-inoculation in chickens infected solely with  $10^4$  *E. praecox* (Ep) oocysts or  $10^4$  *E. maxima* (Emax) oocysts or with  $10^4$  each *E. praecox* and *E. maxima* (Ep + Emax). NI, non-infected controls. Data expressed as mean + S.E.M. of three individual studies. Duncan statistical analysis results are indicated by lower case letters above histogram—no significant difference ( $P > 0.05$ ) between groups that share a letter.

both *E. maxima* and *E. praecox* showed FCR equal to  $3.0 \pm 0.1$  (Fig. 2). Only the group infected solely with *E. maxima* showed a significant increase in FCR relative to the non-infected controls ( $P < 0.05$ ).

### 3.3. Intestinal lesion scores

Average intestinal lesions in chickens infected solely with *E. maxima* oocysts were 1.3, compared to negligible lesions in chickens infected with *E. praecox* alone (Fig. 3). The lesions in *E. maxima*-infected chickens were characteristic of this species with numerous petechiae, thickening of the intestinal wall, and some distension. Consistent with previous observations (Gore and Long, 1982), the only noticeable effect on the upper-middle intestine in *E. praecox*-infected chickens was the presence of a mucoid exudate with moderate bleaching of the intestinal epithelium. A significantly lower average lesion score ( $0.6$ ,  $P < 0.05$ ) was observed in chickens infected with both *E. maxima* and *E. praecox* compared to chickens infected only with *E. maxima* (Fig. 3).

### 3.4. Serum carotenoid levels

Serum carotenoid levels in groups infected with *E. maxima* alone or with both *E. maxima* and *E. praecox* were significantly lower than non-infected controls ( $P < 0.05$ , Fig. 4). Serum carotenoid levels in chickens infected solely with *E. praecox* were significantly higher ( $P < 0.05$ ) than carotenoid levels in chickens infected with *E. maxima* alone or both *E. maxima* and *E. praecox*, but were significantly less than carotenoid levels in non-infected controls ( $P < 0.05$ , Fig. 4).

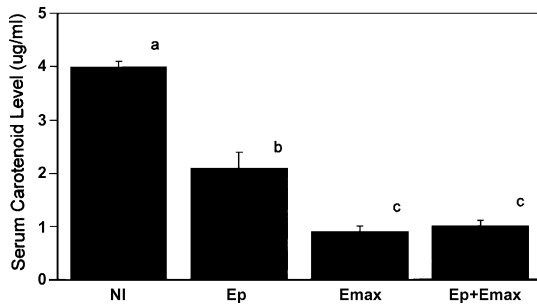


Fig. 4. Mean serum carotenoid levels at 7 days post-inoculation in chickens infected solely with  $10^4$  *E. praecox* (Ep) oocysts or  $10^4$  *E. maxima* (Emax) oocysts or with  $10^4$  each *E. praecox* and *E. maxima* (Ep + Emax). NI, non-infected controls. Data expressed as mean  $\pm$  S.E.M. of three individual studies. Duncan statistical analysis results are indicated by lower case letters above histogram—no significant difference ( $P > 0.05$ ) between groups that share a letter.

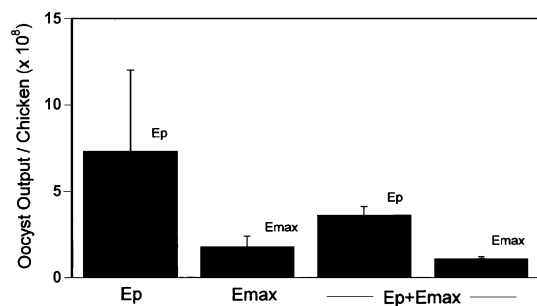


Fig. 5. Mean oocyst output between 3 and 7 days post-inoculation in chickens infected solely with  $10^4$  *E. praecox* (Ep) oocysts or  $10^4$  *E. maxima* (Emax) oocysts or with  $10^4$  each *E. praecox* and *E. maxima* (Ep + Emax). Ep or Emax designations above histogram bar refers to oocyst output for that particular species. Data expressed as mean  $\pm$  S.E.M. of three individual studies.

### 3.5. Oocyst excretion

*E. praecox* oocyst production in chickens infected with both *E. praecox* and *E. maxima* showed a slight, yet insignificant decrease ( $P > 0.05$ ) relative to production in chickens infected solely with *E. praecox* (Fig. 5). A similar pattern was observed with *E. maxima* oocyst production in dual (*E. maxima* and *E. praecox*) or single (*E. maxima*) infections (Fig. 5).

## 4. Discussion

The present study demonstrated that infecting chickens with *E. praecox* can ameliorate several clinical aspects of coccidiosis caused by *E. maxima*. In the absence of *E. praecox*, weight gain is significantly decreased and both intestinal lesion scores and feed conversion ratios are significantly increased in chickens

infected with *E. maxima* alone. Dual infection with both *E. praecox* and *E. maxima* provides significant ( $P < 0.05$ ) protection against weight gain depression and lower feed efficiency compared to infection with *E. maxima* alone. The intensity of *E. maxima*-type intestinal lesions was lower in dual infections, but showed no significant difference from those observed in a single *E. maxima* infection. The only parameter that appears to be affected by *E. praecox* is serum carotenoids, which showed a significant decrease compared to uninfected controls. Serum carotenoids in chickens infected with *E. praecox* alone was intermediate to carotenoid levels in single *E. maxima* or dual *E. praecox*–*E. maxima* infections. These data suggest that *E. praecox* does not prevent carotenoid malabsorption associated with *E. maxima* infection. These results are not entirely consistent with another study that found no decrease in serum carotenoids during *E. praecox* infection (Marusich et al., 1973). The differences may be due to the strain of *E. praecox* used or to carotenoid-supplemented feed utilized in the study.

The lower pathogenicity of *E. praecox* is consistent with previous observations (Long, 1968; Gore and Long, 1982; Jorgensen et al., 1997; Williams, 1998). Similar to these reports, the present study indicates minimal intestinal lesions arising from *E. praecox* infection. The mid-upper intestine in *E. praecox*-infected chickens displayed a typical mucoid watery exudate, with some bleaching of the intestinal wall. It is unclear how *E. praecox* can prevent weight gain depression and lower feed efficiency associated with *E. maxima*. This phenomenon is probably related to a number of factors, including the faster rate at which *E. praecox* excysts (unpublished observations), and the shorter patency and higher fecundity of *E. praecox* relative to *E. maxima*. Another possible factor may be the more rapid rate of migration to and invasion of the gut mucosa by *E. praecox* (Fernando et al., 1987). Recent studies in our laboratory have found a peak serum nitrite/nitrate levels in *E. praecox*-infected chickens at 4 days post-infection compared to 6 days post-infection in *E. maxima*-infected chickens (P. Allen, personal communication). It is possible that this non-specific cellular response may affect certain clinical features (e.g. weight gain, FCR) of an ongoing *E. maxima* infection. In preliminary studies, we found no effect of *E. praecox* on either *E. acervulina* or *Eimeria tenella* infections (unpublished observations). The positive effect of *E. praecox* on clinical features of *E. maxima* infection suggests that these two species invade similar regions of the small intestine, and that non-specific immunity elicited by the former may affect

the latter. An avenue of research that we are pursuing is the effect of dual infection with *E. praecox* and *E. maxima* on immunity that develops against either species arising from a single *E. praecox* or *E. maxima* infection. However, the presence of *E. praecox* on poultry farms that have used live oocyst vaccines containing *E. maxima* suggests that there is little cross-immunity between these two species (Morris et al., 2007).

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